PROPOSAL FOR TOPIC APPROVAL TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR THE PH.D. DEGREE IN THE SUBJECT FOR PHARMACOGNOSY.

Title of the topic:	**Evaluation of polyherbal formulation prepared with improved approach**
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1. Title : Evaluation of polyherbal formulation prepared with improved approach

2. Aim of study:

The aim of study is to formulate, characterize and evaluate the cardioprotective efficacy of iron oxide nanoparticles loaded with polyherbal formulation containing the extracts of *Terminalia arjuna, Terminalia chebula, Trapa bispinosa, Phyllanthus emblica, Tribulus terrestris, Sida cordifolia, Withania somnifera, Glycerrhiza glabra, Terminalia belerica, Hemidesmus indicus, Tinospora cordifolia, Commiphora mukul, Mineral pitch (Shilajit).*

3. Objectives of study:

- Procurement, authentication and processing of plant materials
- Preliminary physicochemical analysis of powdered crude drugs
- Preparation of plant extracts and their preliminary phytochemical analysis
- Preparation of polyherbal formulation
- Standardization, quantification, method development and validation of polyherbal formulation using marker compounds
- Synthesis and characterization of the iron oxide nanoparticles loaded with polyherbal formulation
- Evaluation of iron oxide nanoparticles loaded with polyherbal formulation
- Bio-evaluation of iron oxide nanoparticles loaded with polyherbal formulation

4. Study Background (Literature Review) and significance of study :

Cardiovascular diseases (CVDs) affect many millions of individuals every year and they are the leading cause of death globally, with an estimate of 31% [1,2]. CVDs are progressive and complex diseases and include cardiomyopathies, ischemic heart disease, thromboembolic disease and finally heart failure. Hypercholesterolemia and diabetes are risk factors for CVDs. Overall incidence of these risk factors is increasing in the population. Moreover, Cardiovascular system (CVS) related conditions and disorders are seeking attention during the time of COVID-19 due to the surge in fatalities reported with cardiovascular comorbidities[3]. It is reported that in the year after recovering from the acute phase of Covid 19 infection, patients had increased risks of an array of cardiovascular problems, including heart muscle inflammation, blood clots, strokes, myocardial ischaemia , and heart failure[4]. Studies have reported both, the adverse outcomes due to underlying CVD and the viral infection itself, may be responsible or is associated with complications of CVS in COVID-19 positive individuals[3]. Medical therapy for heart failure as well as hypertension, which is a CVD risk factor includes beta blockers, Angiotensin Converting Enzyme inhibitors, Angiotensin Receptor Neprilysin Inhibitor, Angiotensin Receptor Blocker and diuretics. It needs to be noted that long term adverse effects of these drugs include dry cough, fatigue , dizziness, kidney dysfunction, hyperkalaemia, lower WBC counts.

The use of medicinal herbs continues to be an alternative treatment approach for several diseases including CVDs. Currently, there is an unprecedented drive for the use of herbal preparations in modern medicinal systems. This drive is powered by several aspects, prime among which are their cost-effective therapeutic promise compared to standard modern therapies and their safety profile. Herbal remedies have multimodal mechanism of action, such as antioxidant, vasorelaxant, anti-inflammatory effects. They are reported to inhibit endothelial dysfunction, platelet activation and lipid peroxidation. Because of a wide range of molecular and cellular targets, herbal preparations can be used to treat and manage CVDs[2].

Nanotechnologies have recognized potential applicability in managing chronic diseases in the past few years, especially CVD[1]. Of particular interest is the use of nanoparticles as drug carriers to increase the pharmaco-efficacy and safety of drug therapies. Advantages of nanoparticles are they result in large surface area to volume ratio, resulting in improved bioavailability; better drug delivery and reduce adverse effects on non-target tissues. They are a promising platform for launching alternative non-conventional therapies [1,5].

Literature survey revealed that herbal drugs - *Terminalia arjuna, Terminalia chebula, Trapa bispinosa, Phyllanthus emblica, Tribulus terrestris, Sida cordifolia, Withania somnifera, Glycerrhiza glabra, Terminalia belerica, Hemidesmus indicus, Tinospora cordifolia, Mineral pitch (Shilajit), Commiphora mukul* have cardioprotective profile.

Pharmacognostic account of herbal drugs [6,7]**:**

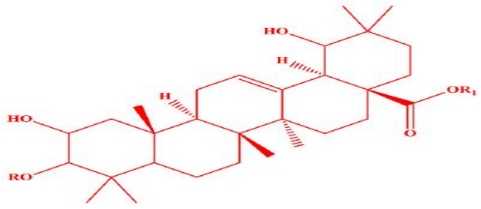
1. ***Terminalia arjuna***

Description	It is widely used herbal plant since ancient times.
Vernacular Names	Arjun, mathi, Neer Maredhu, Aaturmuruthu
Parts Used	Bark
Kingdom	Plantae
Division	Magnoliophyta
• Class	Mangnoliopsida
• Order	Myrtales
• Family	Combretaceae
• Genus	*Terminalia*
• Species	*arjuna*

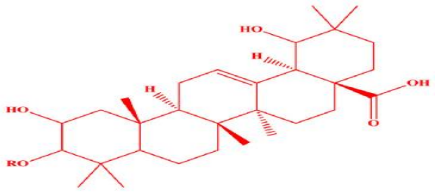
Phytoconstituents[7, 8]**-**

- Triterpenoids - Arjunin, arjunic acid, arjungenin, terminic acid, arjunglucosides I, II, IV and V, arjunosides A-E
- Glycosides – Arjunetin, arjunoside I, arjunoside II
- Flavanoids - Arjunoline, arjunone, baicalein, quercetin, ethyl gallate, quercetin, kempferol, pelargonidin, oligomeric proanthocyanidines

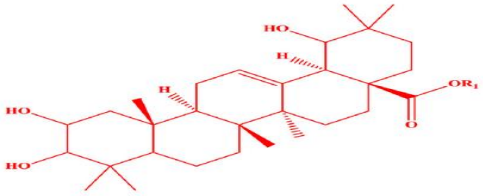
- Tannins: Pyrocatechols, punicallin, punicalagin, terchebulin, terflavin C, castalagin, casuariin and casuarnin
- Polyphenol: gallic acid



1. R = Galactose; R_1 = H; Arjunoside I
2. R = β-D-(+)-glocosyl-L-(-)-2-deoxyrhamanose, R_1 = H; Arjunoside II
3. R = H, R_1 = Gluconic acid; Arjunoside III
4. R = α-L-rhamnopyranose, R_1 = H; Arjunoside IV



1. R = H; Arjunic acid
2. R = β-D-(+)-galactose, Arjunoside I
3. R = β-D-(+)-glocosyl-L-(-)-2-deoxyrhananose, Arjunoside II
4. R = α-L-rhamnopyranose, Arjunoside IV



R1 = Glucose; Arjunetin

Ethnomedical uses[6]:

T. arjuna bark decoction is being used in the Indian subcontinent for anginal pain, hypertension, congestive heart failure, and dyslipidemia, based on the observations of ancient physicians for centuries

Research survey:

- *T. arjuna* bark extract augmented endogenous antioxidant compounds of the rat heart and prevented myocardium from isoproterenol induced myocardial ischemic reperfusion injury[9].
- *T arjuna* bark extract improved the altered myocardial function in diabetic rats possibly through maintaining endogenous antioxidant enzyme activities, decreasing endothelin-1 and inflammatory cytokine levels[10].
- *T. arjuna* bark extract given to patients with stable angina with provocable ischemia on treadmill exercise, led to improvement in clinical and treadmill exercise parameters as compared to placebo therapy[11].
- In patients with coronary heart disease, *T.arjuna* bark powder had significant hypocholesterolaemic effect[12].

2. *Terminalia chebula*

Description	The fruit of the tree possesses diverse health benefits and has been used as traditional medicine since antiquity
Vernacular Names	Haritaki, shilikha; chebulic myrobalan; hardi, harde; hirda
Parts Used	Fruit
Kingdom	Planta
Division	Magnoliophyta
• Class	Magnoliopsida
• Order	Myrtales
• Family	Combretaceae
• Genus	*Terminalia*
• Species	*Chebula*

Phytoconstituents[13]:

Tannins- chebulagic acid, punicalagin, chebulanin, neochebulinic acid, chebulinic acid

Polyphenols - ellagic acid, gallic acid, corilagin, galloyl glucose, punicalagin, terflavin A, maslinic acid

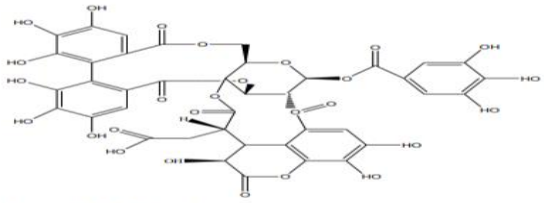


Figure 8. Chebulagic acid.

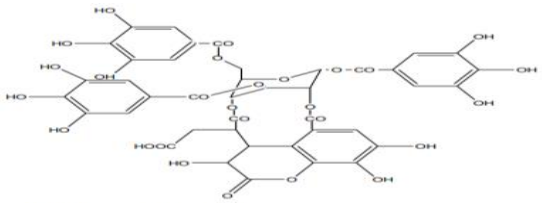


Figure 9. Chebulinic acid.

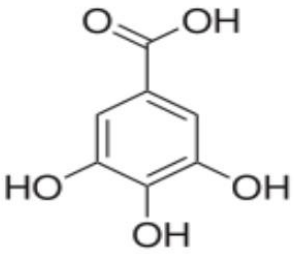


Figure 6. Gallic acid.

Ethnomedical uses[14]:

The fruit is mild laxative, stomachic, tonic. Beneficial in diabetes, skin diseases, heart diseases

Research survey:

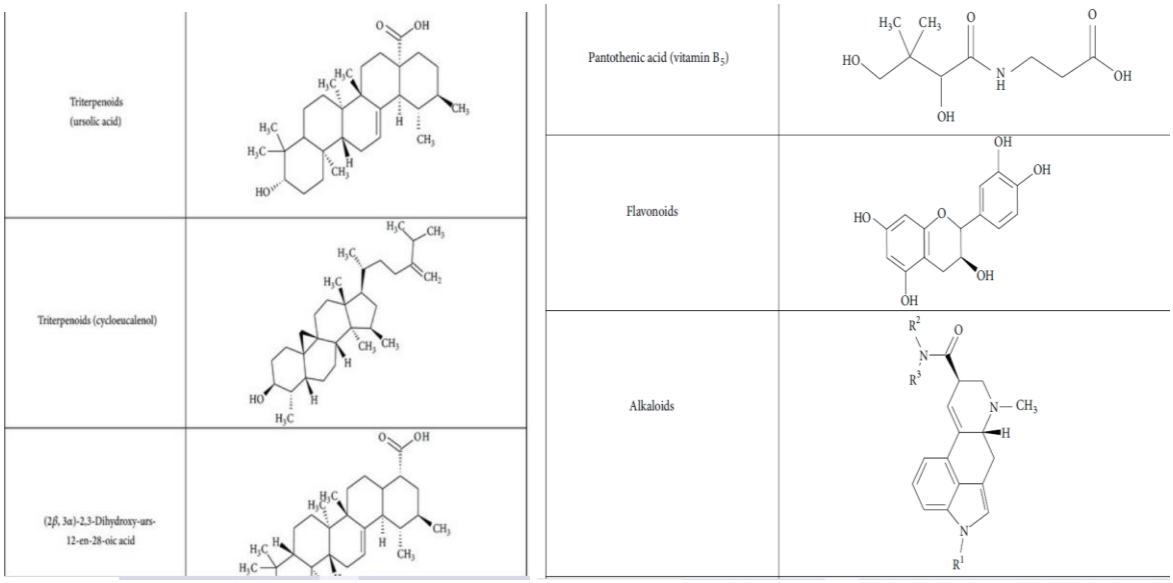
- *T. chebula* extract attenuated rise in lactate, decrease in enzyme activities of tricarboxylic acid cycle (TCA);mitochondrial respiration; levels of adenosine triphosphate (ATP) in isoproterenol induced cardiotoxicity in rats. The results are suggestive of anti-ischemic property of *T. chebula*[15].
- In isoproterenol induced cardiac damage in rats, pretreatment with *T chebula* extract stabilizes the lysosomal membrane and, thus, may have prevented myocardial damage[16].
- *T. chebula* improved endothelial function compared to placebo in type 2 diabetic patients and cardiovascular risk indicators were ameliorated in the treatment groups compared to placebo[17].

3. *Trapa bispinosa*[18]

Description	It is an aquatic plant well known for its medicinal properties and is widely used worldwide
Vernacular Names	Water chestnut, singhara, paniphal, trikonaphala
Parts used	Fruit
Kingdom	Plantae
• Division	Tracheophyta
• Class	Magnoliopsida
• Order	Myrtales
• Family	Trapaceae
• *Genus*	*Trapa*
• *Species*	*bispinosa*

Phytoconstituents[18]:

- Triterpenoids like ursolic acid, cycloeuclenol
- High quantity of minerals, namely, Ca, K, Na, Zn, Flavanoids, alkaloids and vitamins are present.



Ethnomedical uses:

The plant is used as nutritional aid. Has antioxidant and anti-inflammatory activity. It is also used in the problems of stomach, genitourinary system, liver, kidney, and spleen[18],[19]

Research survey:

- In a study done in mice, the ethanolic extract of fruits of *Trapa bispinosa* showed a significant antioxidant activity[19].
- Due to high quantity of minerals, ions, namely, Ca, K, Na, Zn, and vitamins, anti-inflammatory effect is reported.

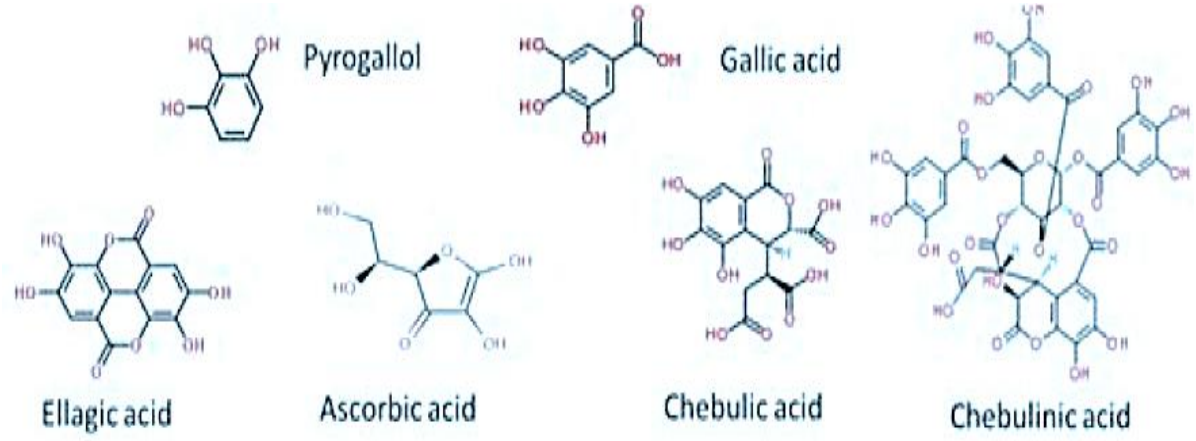
4. *Phyllanthus emblica or Emblica officinalis*

Description	commonly known as Indian gooseberry or *Amla* is one of the most important medicinal plants in Indian traditional systems of medicine
Vernacular names	Amaliki, Amalakan, Sriphalam, Amla , Emblica myroblan
Parts used	Fruit
Kingdom	Plantae
Division	Tracheophyta
• Class	Magnoliopsida
• Order	Malpighiales
• Family	Phyllanthaceae
• Genus	*Phyllanthus*
• Species	*emblica*

Phytoconstituents[20]:

- Tannins - Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corilagin;

- Alkaloids - Phyllantine, Phyllembein, Phyllantidine;
- Phenolic compounds - Gallic acid, Methyl gallate, Ellagic acid;
- vitamin C



Ethnomedical uses :

diuretic, laxative, liver tonic, stomachic, restorative, anti-pyretic, hair tonic, ulcer preventive and for common cold, for strengthening heart

Research survey:

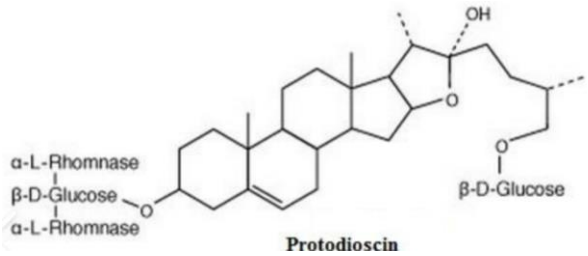
- In a study done in Wistar rats fed with high fat diet, *Emblica officinalis* (EEO) reduced high fat diet induced oxidative stress, indicating its cardioprotective efficacy[21].
- *P. emblica* treatment was shown to be capable of upregulating cardioprotective pathway, thereby preserving cardiac tissue during ischemia-reperfusion injury in a study done on rats[22].
- Treatment with *P. emblica* reduced biomarkers of oxidative stress and systemic inflammation and brought about improvement in endothelial function in patients with type 2 diabetes mellitus[23]

5. Tibulus terrestris

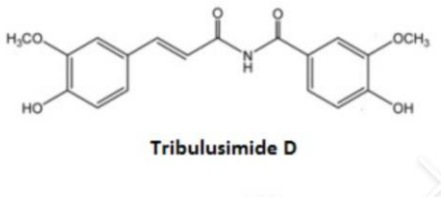
Description	It is an annual herb
Vernacular names	Gokharu, Gokshur, puncture wine
Parts used	Fruit
• Kingdom	Plantae
• Division	Phanerogams
• Class	Magnoliopsida
• Order	Zygophyllales
• Family	Zygophyllaceae
• Genus	*Tribulus*
• Species	*terrestrius*

Phytoconstituents[24]:

- Saponins- protodioscin, protogracillin, diosgenin;
- Flavonoids - quercetin, kaempferol;
- Alkaloids - Tribulusamide D, tribulusterine



Protodioscin



Tribulusimide D

Ethnomedical Uses: tonic, aphrodisiac, palliative, stomachic, antihypertensive, astringent, genitourinary tract disorders. Ayurvedic literature mentions use of this plant for heart diseases

Research Survey:

- *Tribulus terrestris* was found to be effective against cardiac ischemia in rats which was evident from ECG and various cardiac biomarkers analysis. Antioxidant, antiapoptotic and anti-inflammatory potential of *Tribulus terrestris* contributes significantly to its anti-ischemic cardioprotection[25].

- Study in rats showed that Pretreatment with *Tribulus terrestris* showed a protective effect against isoproterenol induced MI (myocardial infarction)[26].

- In diabetic women, total cholesterol and low-density lipoprotein of *T terrestris* group was significantly reduced compared with placebo[27].

6. *Sida cordifolia*

Description	Popularly known as 'bala', the root of Sida cordifolia is regarded as a valuable drug in the Ayurvedic System of Indian Medicine
Vernacular Names	Bala, kharinta, country mellow, chikna
Parts Used	Root
• Kingdom	Plantae
• Division	Tracheophyta
• Class	Magnoliopsida
• Order	Malvales
• Family	Malvaceae
• Genus	*Sida*
• Species	*cordifolia*

Phytoconstituents[28]:

- Alkaloids - β-phenethylamines, carboxylated tryptamines, quinazoline alkaloids; vasicine , vasicinone , and vasicinol
- Flavonoids - 5,7-dihydroxy-3- isoprenyl flavones and 5-hydroxy-3-isoprenyl flavones
- Phytoecdysteroids - sidasterone A and sidasterone B

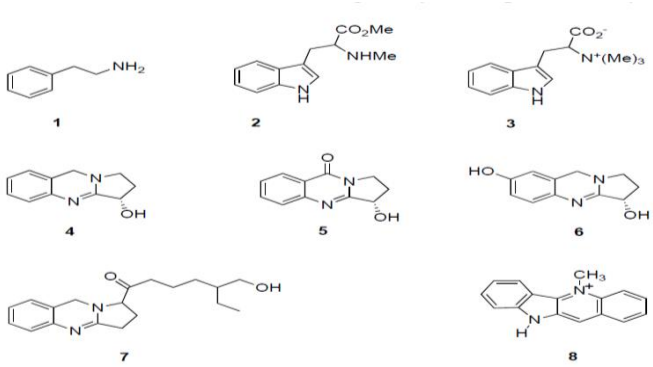


Fig. (1). Alkaloids in *S. cordifolia*. **1:** *β*-phenethylamine; **2**: S-(+)-N_b-methyltryptophan methyl ester; **3:** hypaphorine; **4**: vasicine; **5:** vasicinone; **6:** vasicinol; **7:** 5`-hydroxymethyl-1`-(1,2,3,9-tetrahydro-pyrrolo [2, 1-b] quinazoline-1-yl)-hepta-1-one); **8**: cryptolepine.

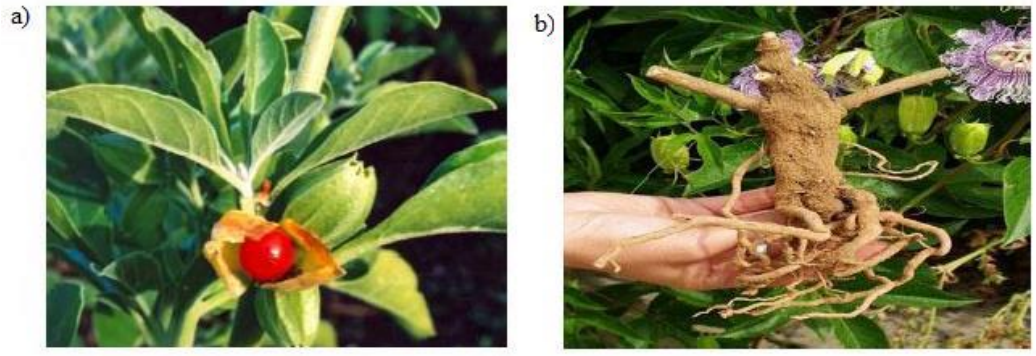
Ethnomedical uses :

It is used as antirheumatic, analgesic, antipyretic, antiasthmatic, nasal decongestant, antiviral and to treat cardiovascular complaints

Research survey:

- *Sida cordifolia* possesses potent protective action against isoproterenol and ischemia reperfusion injury induced myocardial damage in rats[29].
- *Sida cordifolia* was evaluated by in vitro and in vivo experimental models such as clot lysis assay, acute pulmonary embolism, carrageenan-induced tail vein thrombosis and ferric chloride-induced carotid arterial thrombosis. The results demonstrated the antithrombotic and thrombolytic potential of *Sida cordifolia* [30].

7. *Withania somnifera*

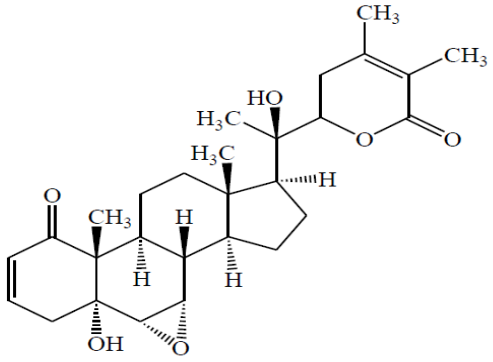


Description	It is a woody shrub regarded as a valuable drug in the Ayurvedic System of Indian Medicine

Vernacular names		Ashwagandha, Indian Ginseng, Winter cherry, Asvagandha, Aksand
Parts used		Root
i)	Kingdom	Plantae
ii)	Division	Tracheophyta
iii)	Class	Magnoliopsida
iv)	Order	Solanales
V)	Family	Solanaceae
v)	Genus	*Withania*
vi)	Species	*somnifera*

Phytoconstituents[31]:

- Alkaloids - somniferine, somnine, somniferinine, withamine, withanmine;
- steroidal lactones named withanolides (eg. Withanolide A),
- saponins, phenolics, flavonoids, phytophenols, and glycosides.

Withanolides	Chemical name	Structure
Withanolide A	(20R)6a,7a-Epoxy 5a,20bdihydroxy-1-oxowitha-2,24-Dienolide	

Ethnomedical uses:

The plant is used to treat several diseases such as fever, cancer, asthma, diabetes, ulcer, hepatitis, eyesores, arthritis and heart problems.

Research survey:

1. *Withania somnifera* exerts a strong cardioprotective effect in the experimental model of isoprenaline-induced myonecrosis in rats. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered haemodynamic parameters may contribute to its cardioprotective effect[32].

2. In Wistar rats, doxorubicin-induced biochemical and histological alterations were effectively attenuated by prior administration of *Withania somnifera* extract, suggesting a role of *Withania somnifera* in protection against cardiotoxicity induced by doxorubicin [33].

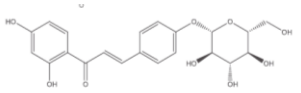
8. *Glycerrhiza glabra*

Description	It is a typical herbaceous perennial
Vernacular Names	Licorice, jethi-madh, kubas-susa, mithilakdi Jeshtamadhu,Yastimadhu, Atimadhura, atimadhuram, erattimadhuram , jashtimadh
Parts Used	Root
Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	*Glycerrhiza*
Species	*glabra*

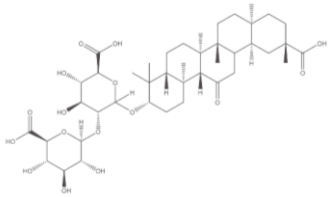
Phytoconstituents[34]:

- Triterpenoid saponin - Glycyrrhizin
- Flavonoids: liquiritigenin (4′,7-dihydroxyflavanone) and isoliquiritigenin.

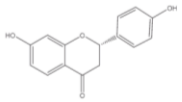
Isoliquiritin



Glycyrrhizin



Liquiritigenin



Ethanomedical uses:

Glycyrrhiza glabra L. (Licorice) is traditionally used to treat many diseases, such as respiratory disorders, epilepsy, fever, stomach ulcers, rheumatism, skin diseases, jaundice. It is reported to be good for heart health. Prevents damage from LDL cholesterol and plaque formation. It shows antioxidant, anti-inflammatory property.

Research survey:

- In isoproterenol (ISP)-induced myocardial injury in rats, Licorice treatment exerted cardioprotection by reducing oxidative stress, augmenting endogenous antioxidants, and restoring functional parameters as well as maintaining structural integrity[35].
- Study done on *Glycyrrhiza glabra* against ischemia–reperfusion induced injury in rats suggested cardioprotective potential of G. glabra against myocardial infarction by amelioration of oxidative stress and favourable modulation of cardiac function[36]

9. *Hemidesmus indicus*

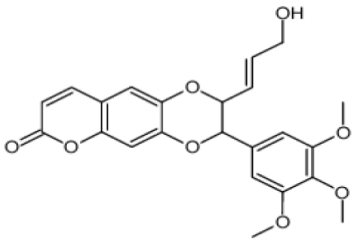
Description	It is a vine, which trails on the ground and climbs by means of tendrils.
Vernacular names	Indian Sarsaparilla, Anantmul , Anantvel
Parts used	Root
• Kingdom	Plantae
• Division	Tracheophyta
• Class	Magnoliopsida

- Order Gentianales
- Family Asclepiadaceae
- Genus *Hemidesmus*
- Species *indicus*

Phytoconstituents[37]**:**

- Alkaloids: hemideminine
- Steroid: beta sitosterol, hemidosterol, hemidesmol
- flavonoids, phenolic compounds, and tannins

Hemidesminine



Ethnomedical uses:

Treating snakebites, scorpion, diabetes, urinary diseases, anti-atherogenic, anti-spasmodic, memory enhancing, immune-potentiating and anti-inflammatory

Research survey:

- Cardioprotective effects of *H. indicus* are suggested in Langendorff-perfused rat hearts with induced ischaemia[38].
- *H. indicus* protects against oxidative stress, hyperlipidemia against High Fat Diet - induced oxidative stress in rats[39] .

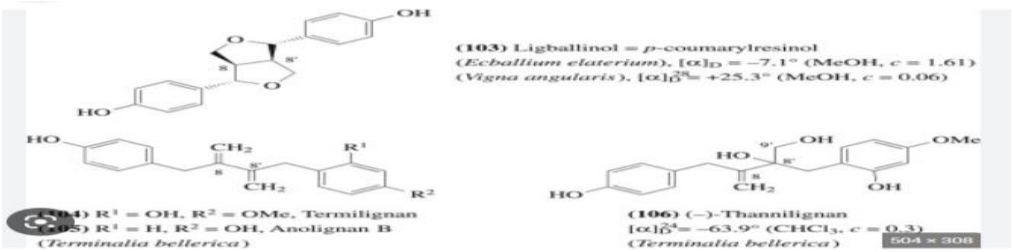
10. Terminalia bellerica

Description It is a large deciduous tree

Vernacular names	Vibheetaki Bahera, Baheda, Bibhitaki, Belleric Myrobalan, Bedda Nut Tree, Beach- Almond
Parts used	Fruit
• Kingdom	Plantae
• Division	Tracheophyta
• Class	Magnoliopsida
• Order	Myrtales
• Family	Combretaceae
• Genus	*Terminalia*
• Species	*bellerica*

Phytoconstituents[40]**:**

- Polyphenols- Gallic acid, ellagic acid, methyl gallate, ethyl gallate
- Lignans – Thannilignan
- Steroids – beta-sitosterol
- Flavanoids - Quercetin and kampferol


(103) Ligballinol = p-coumarylresinol
(Ecballium elaterium), [α]D = −7.1° (MeOH, c = 1.61)
(Vigna angularis), [α]D = +25.3° (MeOH, c = 0.06)
(106) (−)-Thannilignan
(Terminalia bellerica)
504 × 308

Research survey:

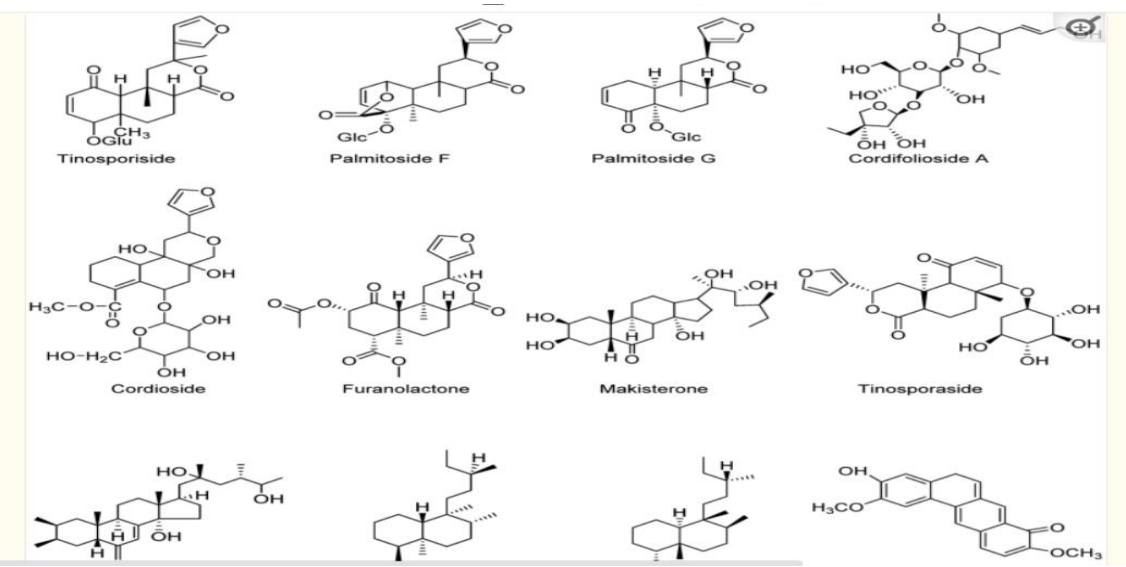
- *T. bellerica* reduced the levels of lipids in hypercholesterolemic rabbits [41].
- A broad spectrum in vitro and in vivo animal studies suggested various effects of *T.bellerica*, including antioxidant, anti-inflammatory effects[42].

11. Tinospora cordifolia

Description		It is a deciduous climbing shrub, designated as Rasayana in Ayurveda.
Vernacular names		Giloy, Guduchi, Amrita
Parts used		Root
i)	Kingdom	Plantae
ii)	Division	Magnoliophyta
iii)	Class	Magnoliopsida
iv)	Order	Ranunculalaes
V)	Family	Menispermaceae
v)	Genus	*Tinospora*
vi)	Species	*cordifolia*

Phytoconstituents[43]

- Terpenoids - Tinosporide, Furanolactone diterpene, Furanolactone clerodane diterpene, furanoid diterpene
- Alkaloids -Tinosporine, Magnoflorine, Berberine, Choline, Jatrorrhizine



Ethnomedical uses: The plant is commonly used as traditional Ayurvedic medicine and has several therapeutic uses such as in jaundice, rheumatism, heart diseases associated with pain, urinary disorder, skin diseases, diabetes, anemia, inflammation, allergic condition

Research survey:

- *Tinospora cordifolia* effectively prevented the altered levels of serum marker enzymes (creatine kinase and lactate dehydrogenase), antioxidants, glycoproteins contents by Cadmium (Cd) intoxication. The study suggests *Tinospora cordifolia* as a potent cardioprotective agent against Cd induced toxicity[44]
- The study suggests cardioprotective activity of *T. cordifolia* in ischemia-reperfusion induced myocardial infarction in rats[45].

12.Commiphora mukul (Commiphora wightii)

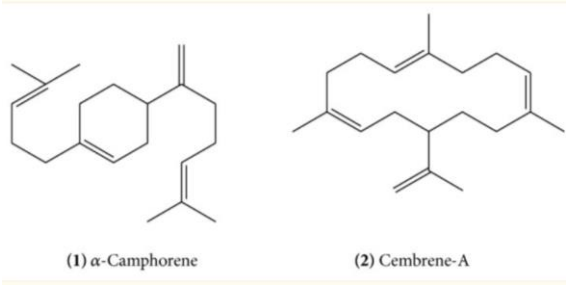
Description Plant is a Woody shrub or small tree

Vernacular names	Myrrh tree, Mukul, Indian bedellium, Guggulu, Guggal
Parts used	Gum guggal
i)Kingdom	Plantae
ii)Division	Tracheophyta
iii)Class	Magnoliopsida
iv)Order	Sapindales
v)Family	Burseraceae
vi)Genus	Commiphora
vii)Species	mukul

Ethnomedical Uses : The extract of gum guggal, called gugulipid has been used in Unani and Ayurvedic medicine, for many centuries . It is used to treat inflammation, gout, rheumatism, obesity and disorders of lipid metabolism.

Phyto*constituents*[46]-

- diterpenoids - α-camphorene, cembrene-A **;**
- Steroids- Guggulsterone.
- It also contains long-chain aliphatic tetrols, aliphatic esters, ferulates, lignans, carbohydrates, and a variety of inorganic ions



(1) α-Camphorene (2) Cembrene-A

Research survey:

- In isoprenaline-induced myocardial necrosis in rats, cardioprotective effects of *Commiphora mukul* were shown[47].
- *C. mukul* significantly improved the cardiac function and prevented myocardial ischemic impairment in isoproterenol-induced myocardial infarction in rats[48].

13.Mineral Pitch-Shilajit (Asphaltum Punjabianum)

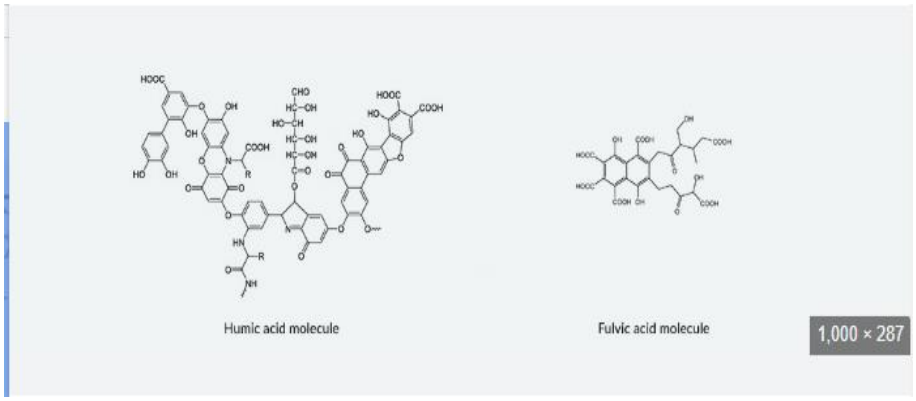
Description	Shilajit is a multi-component natural occurring mineral substance used in Ayurveda and Siddha systems of medicine
Common names	Mineral pitch, vegetable asphalt, bitumen , Jew's pitch,; Silajatu, mumiyo

There is no further taxonomic classification for Shilajit. It is simply listed as a "drug of mineral origin" in the Ayurvedic Formulary. Research at Banaras Hindu University in India reveals via chemical analysis that Shilajit is the result of the humification of resin and latex bearing plants including *Euphorbia royleana* and *Trifolium repens*.

Ethnomedical uses: It is referred as 'rasayana'/'rasayanam' in Ayurveda and Siddha literature which means rejuvenator because it prevents ailment and enhances the quality of life. Shilajit has been used in nervous, diabetic, immune, cardiac, asthma, joint disorders.

Phytoconstituents:[49]

- Resins, Benzoic acid, hippuric acid, fulvic acid, humic acid
- Minerals: silica, iron, antimony, calcium, copper, lithium, magnesium, manganese, molybdenum, phosphorus, sodium, strontium, zinc



Research Survey:

- Prominent cardioprotective effect of Shilajit against destructive effects of isoproterenol were reported in a study done in rats[50].
- Various research studies indicate that Shilajit exhibits antioxidant, anti-inflammatory and anti-dyslipidemic properties[49].

5. International Status

Mohamed N. A. from Qatar University, Doha along with Marei I from Imperial College, London and other authors reported that nanotechnologies have recognized potential applicability in managing chronic diseases in the past few years, especially CVDs [1].

Hesari M from Kermanshah University of Medical Sciences, Iran along with other authors reported in a review article that " in the context of CVDs prevention and/or treatment, available data suggests that natural-based nanoformulations are considerably efficient, alone or in blend with other herbal/synthetic medicines [51]".

Abert H A from Modibbo Adama University, Nigeria along with other authors demonstrated that iron oxide nanoparticles of a herbal extract were effective in ameliorating Triton X-100-induced atherosclerosis in rats, suggesting its cardioprotective action [52].

Kiani B H from Islamabad, Pakistan along with other authors have reported preparation of zinc nanoparticles of *Withania somnifera*[53]. Wang R at college of life sciences, Korea along with other authors have formulated gold nanoparticles of *Phyllanthus emblica*[54]. Preparation of mucoadhesive nanoparticles of *Glycerrhiza glabra* has been reported by Roque L at Lisboa, Portugal and other authors[55].

Hence, it can be seen that there has been research conducted Internationally on herbal based nanoparticles and their cardioprotective action.

6. National Status

Teja Parusu Kavya from National Institute of Pharmaceutical Education and Research-Ahmedabad (NIPER-A) along with other authors reported that herbal nanomedicines are safer, have higher bioavailability, and have enhanced therapeutic value than conventional herbal and synthetic drugs [56].

Vikas Murkute from AISSMS College of Pharmacy, Pune along with other authors reported that " Instead of using monotherapy, combination therapy or polyherbal combination can be a better option to manage cardiovascular disease[57]".

Balliah R from Department of Biochemistry, PSG College of Arts and Science, Coimbatore, reported that in isoproterenol induced myocardial infarction study in Wistar albino rats, polyherbal crude extract *was shown to* exhibit the cardioprotective activity[58].

Pallela P N at advanced analytical laboratory, Vishakhapatnam along with other authors reported formulating iron nanoparticles of *Sida cordifolia*[59].Ankegowda V M at Bangalore Institute of Technology along with other authors reported formulating silver nanoparticles of *T. chebula* fruit extract[60]. Ahmed Q at central university of Rajasthan, Ajmer along with other authors have formulated silver nanoparticles of *T. arjuna* bark extract[61]

Brito Raj S at Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh have reported synthesis of silver nanoparticles from a polyherbal formulation [62]. J. Sasidharan at Trichy along with other authors have reported formulating silver and iron nanoparticles from a polyherbal formulation [63]. Hemalakshmi. M and P. Chitra at Sri Ramakrishna college of Arts and Science for Women, affiliated to Bharathiar University Coimbatore, TamilNadu have reported synthesis of silver nanoparticles using polyherbal formulation[64]. Eswaran A at Department of Biochemistry, Periyar University, Salem, Tamil Nadu, India along with other authors have reported synthesis of silver nanoparticles from polyherbal formulation[65]

Hence, it can be seen that there has been research conducted in India on formulating herbal as well as polyherbal nanoparticles. Polyherbal combination is suggested to be a better option than monotherapy in managing cardiovascular disease.

6. Study hypothesis

The use of medicinal herbs continues to be an alternative treatment approach for several diseases including CVDs. Phytoconstituents present in some plants possess significant cardioprotective property. Also, natural products have fewer adverse effects than synthetic drugs. It is reported that polyherbal formulations give synergistic effect[57]. Instead of using monotherapy, combination therapy or polyherbal combination can be a better option to manage cardiovascular disease[57.] The same is hypothesised in context of a polyherbal formulation (Collasyn tablets, marketed by Dr. Palep Research Laboratories) claimed to be cardioprotective.

Recently nanotechnology is playing an important role in the area of CVD management[1]. It is reported that herbal nanomedicines have enhanced therapeutic value than conventional herbal and synthetic drugs[56].

Hence, with this hypothesis, it is thought important to formulate, characterize and evaluate the cardioprotective activity of IONPs (Iron Oxide Nano Particles) loaded with polyherbal formulation, (prepared by taking the same herbs in the given quantities as in Collasyn tablets), containing the extracts of *Terminalia arjuna, Terminalia chebula, Trapa bispinosa, Emblica officinalis, Sida cordifolia, Withania somnifera, Glycerrhiza glabra, Hemidesmus indicus, Tribulus terrestris, Terminalia bellerica, Tinospora cordifolia, Commiphora mukul, Mineral pitch*

However, a study of such kind on polyherbal nanoparticles in management of CVD has not been reported to the best of our knowledge after screening the literature. If synthesis and stability of iron oxide nanoparticles loaded with polyherbal formulation, (prepared by taking the same herbs in the given quantities as in Collasyn tablets) is not achieved, it is thought important to formulate, characterize and evaluate the cardioprotective activity of IONPs loaded with two or three major herb extracts as in Collasyn tablets or a polyherbal phytosome.

7. Study Rationale

Cardiovascular diseases (CVDs) are the leading causes of death worldwide. The same holds true in spite of preventive, diagnostic and treatment measures being used. Herbal formulations have been used as a very good alternative for conventional treatments.

Herbal remedies have multimodal mechanism of action, such as antioxidant, vasorelaxant, anti-inflammatory effects. Because of a wide range of molecular and cellular targets, herbal preparations can be used to treat and manage CVDs[2]. The therapeutic effect of herbal medicines are exerted due to the presence of different phytoconstituents and the effects are further potentiated when compatible herbals are formulated together. Often, Polyherbal Formulations result in fewer side effects as compared to allopathic drugs[57].

It is reported that herbal nanomedicines are safer, have higher bioavailability, and have enhanced therapeutic value than conventional herbal and synthetic drugs[56]. In the context of CVDs prevention and/or treatment, available data suggests that natural-based nanoformulations are considerably efficient, alone or in blend with other herbal/synthetic medicines[51].

Collasyn tablet marketed by Dr. Palep Research Laboratories is a polyherbal formulation, claimed to be cardioprotective prepared on the basis of ethnomedical usage of different herbs. However, there is no bio-evaluation data available on the product. Therefore, this research aims at generating scientific evidence on cardioprotective efficacy of this polyherbal formulation and its iron oxide nanoparticles. Hence, it is thought worthwhile, taking the same herbs in the given quantities as in Collasyn tablets, to formulate, characterize and evaluate the cardioprotective activity of IONPs (Iron Oxide Nano Particles) loaded with polyherbal formulation (containing the extracts of *Terminalia arjuna, Terminalia chebula, Trapa bispinosa, Emblica officinalis, Sida cordifolia, Withania somnifera, Glycerrhiza glabra, Hemidesmus indicus, Tribulus terrestris,Terminalia bellerica, Tinospora cordifolia, Commiphora mukul, Mineral pitch-Shilajit).*

8. **Research Methodology**

8.1 Procurement, authentication and processing of the plant material

Barks of *Terminalia arjuna,* Fruits of *Terminalia chebula,* Fruits of *Trapa bispinosa,* Fruits of *Phyllanthus emblica ,* Roots of *Sida cordifolia,* Roots of *Withania somnifera,* Roots of *Glycerrhiza glabra,* Roots of *Hemidesmus indicus,* Fruits of *Tribulus terrestris,* Fruits of *Terminalia bellerica,* Roots of *Tinospora cordifolia,* Gum guggul of *Commiphora mukul,* Mineral pitch (Shilajit) will be obtained from Dr. Palep's research laboratory as gift samples and authenticated on the basis of microscopic and morphological analysis. Respective parts will be dried and powdered.

8.2 Preliminary physicochemical analysis of powdered crude drugs

The crude drug powders will be evaluated for parameters like total ash, acid insoluble ash, foreign matter, moisture content, extractive values. The standard procedures mentioned in Indian Herbal Pharmacopoeia will be adapted.

8.3 Preparation of plant extracts and their preliminary phytochemical analysis

Each powdered crude drug will be extracted with hydroalcoholic (ethanol : water -7:3) and with water as a solvent. The plant extracts will undergo ultrasonication. The extracts will be filtered. These extracts will then be subjected to preliminary phytochemical analysis using standard tests to detect various types of phytoconstituents..

8.4 Preparation of polyherbal formulation

By taking same quantities of plant extracts as in Collasyn tablet, marketed by Dr. Palep research laboratories, a polyherbal formulation will be prepared.

8.5 Standardization, quantification, method development and validation of polyherbal formulation using marker compounds

Standardization of polyherbal formulation will be done by using UV and TLC. Marker compounds will be used for standardization. TLC and HPLC studies will be done to decide on which marker compound needs to be used. Mobile phase would be chosen depending upon the nature of the marker compound. Subsequent quantification of polyherbal extract will be done by HPLC. The analytical method development would be done using HPLC for the simultaneous estimation of the active biomarkers in the formulation.

8.6.1 Validation of the analytical method

This would be done according to ICH guidelines. The parameters include accuracy, precision, limit of detection, limit of quantification, linearity, range, robustness.

8.7 Synthesis and characterization of Iron Oxide Nanoparticles loaded with polyherbal formulation

Iron Oxide Nanoparticles loaded with polyherbal formulation will be synthesized by optimizing conventional methods. The characterization of the Iron Oxide Nano Particles (IONPs)will be carried out using UV-vis spectroscopy, X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

8.8 Evaluation of the iron oxide nanoparticles of polyherbal formulation

Prepared nanoparticles would be evaluated for parameters like yield of Nanoparticles, Drug content / surface entrapment / drug entrapment, particle size and zeta potential, surface morphology, Polydispersity index, in-vitro release study, stability study of nanoparticles

8.9 Bio-evaluation of the polyherbal nanoparticles for therapeutic activity

In vitro study

Tryptan Blue exclusion test of cell viability

Different quantities of the iron oxide nanoparticles loaded with polyherbal formulation and polyherbal formulation will undergo Trypan Blue exclusion test of cell viability using H9c2-Rat Cardiomyocyte cell line.

Cells will be pre-treated with various concentrations of iron oxide nanoparticles of polyherbal formulation and polyherbal formulation prior to the induction of ischemia .To induce ischemia, the cells will be maintained in an ischemic buffer (composition in mM -137 NaCl, 12 KCl, 0.5 MgCl2, 0.9 CaCl2, 20 HEPES, 20 2-deoxy-d-glucose, pH-6.2) at 37°C with 0.1% O2, 5% CO2, and 95% N2 in a hypoxia incubator for 1hour.The analysis will be estimated by cell viability assay using Trypan Blue dye exclusion method and a microscopic monitoring of the morphology of the cells.

Negative control (medium with cells but without the experimental drug/compound) and Positive control (Medium with cells and Cyclosporin A – 1 micromole) followed by Ischemic buffer-1hr as well as Treatment control (Cells pre-treated with various concentrations of iron oxide nanoparticles of polyherbal formulation and polyherbal formulation without ischaemic buffer).

The percentage viability will be plotted against the quantity of formulation used. The percentage of viable cells will be calculated as follows :

% viability = Number of viable cells / Total number of cells X 100

ATP, which is a mitochondrial function parameter will be measured in cells.

In vivo study - Animal study

The cardioprotective effects will be evaluated in isoproterenol induced cardiotoxicity (myocardial ischemia) in rats. Iron oxide nanoparticles loaded with polyherbal formulation and polyherbal formulation will be tested in this study. Male wistar albino 10-12 weeks old rats weighing 180 g- 230 g will be used in study. Animals will be divided in groups of 6 rats each.

Group I – Healthy control (orally administered feed +water)

Group II – Disease (Ischaemic) control (Isoproterenol)

Group III – Standard control - propranalol + isoproterenol

Group IV – Treatment control - polyherbal formulation

Group V- Treatment control- Iron oxide nanoparticles loaded with Polyherbal formulation

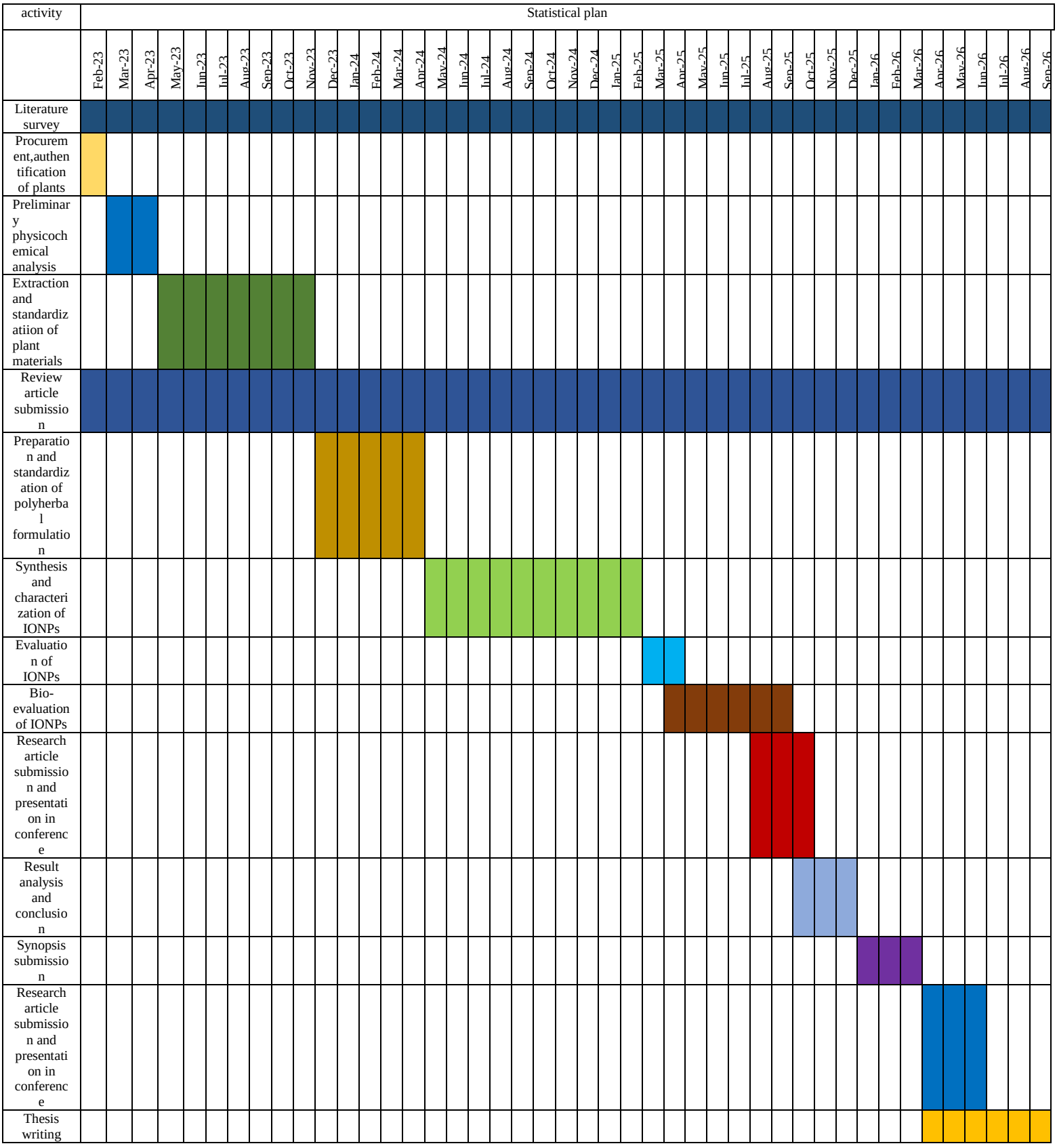
Group VI - Polyherbal formulation + Isoproterenol

Group VII- Treatment - Iron oxide nanoparticles loaded with Polyherbal formulation + Isoproterenol

Groups IV to VII, will be administered test drug orally for 30 days. Standard orally administered feed + water will be given to all groups. Myocardial ischaemia will be produced in rats using isoproterenol subcutaneous injection on day 28^{nd} and 29^{th} at an interval of 24 hours in groups II to VI. Levels of cardiac biomarker CK-MB (Creatine kinase -Myocardial B fraction) , Troponin I , LDH (Lactate dehydrogenase) will be studied in serum . Antioxidants– superoxide dismutase / reduced glutathione will be studied in serum. Blood samples of 12 hour fasted rats will be collected from retro orbital plexus at 0^{th} , 21^{st} and 30^{th} day to perform biochemical studies. ECG and blood pressure measurements will be done. After blood sampling on 30^{th} day, rats will be anaesthetized with anaesthetic ether, then sacrificed and the hearts will be harvested for histological studies.

In treatment group, reduction in serum levels of cardiac biomarkers and increase in antioxidants will be seen as compared to disease group.

9. Statistical plan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Statistical plan
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Literature survey
Procurement,authentification of plants
Preliminary physicochemical analysis
Extraction and standardizatiion of plant materials
Review article submission
Preparation and standardization of polyherbal formulation
Synthesis and characterization of IONPs
Evaluation of IONPs
Bio-evaluation of IONPs
Research article submission and presentation in conference
Result analysis and conclusion
Synopsis submission
Research article submission and presentation in conference
Thesis writing

10. Limitations

The synthesis and full characterization of herbal nanoparticles is challenging. The properties of nanoparticles are dependant on method of synthesis and characterization. Method of synthesis needs to be studied and executed well . That will be guider to produce well defined particles. Research reports that it is very difficult to predict amount of extract trapped in nanoparticles.

11. Expected outcomes of the study

Cardiovascular diseases, the leading cause of death globally, are seeking higher attention during the time of COVID-19 due to the surge in fatalities reported with cardiovascular comorbidities[3]. There is urgent and unmet need to develop novel and efficacious cardioprotective medicine for management of CVDs. Recently nanotechnology is playing an important role in the area of CVD management[1]. It is reported that herbal nanomedicines have enhanced therapeutic value than conventional herbal and synthetic drugs[52]This study will generate scientific evidence on cardioprotective efficacy of iron oxide nanoparticles loaded with a polyherbal formulation (prepared by taking the same herbs in the given quantities as in Collasyn tablets, marketed by Dr. Palep Laboratories).Hence, the proposed research study will be a crucial step in developing polyherbal medicine using nanoparticle technology for management of CVDs.

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